a liquid onto a selected portion of the surface of a substrate including all elements as described in claim 1 on or before September 17, 1998. Exhibit A is attached hereto in support of the declaration. Exhibit A was provided to P.J. van der Schaaf at the Delft University of Technology on August 13, 1998 by Applicant as an invention disclosure describing an invention described in claim 1 that had been reduced to practice by Applicant prior to August 13, 1998.

Therefore, Moerman conceived and invented the claimed subject matter before the prior art date of Moon (September 17, 1998). This is sufficient to overcome a §103(a) rejection based on the Moon *et al.* reference.

Respectfully submitted,
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CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Mail Stop Issue Fee, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on this 22 day of March, 2005.

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Name

Signature

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Exhibit A

Patent afdeling (SBS):
P.J. van der Schaaf@bu.tudelft.nl

TU Delft, TNW, Bioprocestechnology
DIOC-5-sampling program (IMDS Intelligent Molecular Diagnostic Systems)
Program leader : Prof. Dr. Ian T. Young (technische natuurkunde)
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ABSTRACT

The invention relates to the injection of liquids in sub-nanoliter wells of a micro-array with flowrates lower than 50 picoliters per second. The method is based on the electrospray principle with which it is proven to be possible to inject samples with flowrates down to 1.0 nanoliter per minute or less (WO 98/08613). On decreasing the distance between the injection capillary and the surface it is possible to reduce the spray diameter to 200 micrometer or less. The new injection technique can be used to inject picoliter volumes of liquids containing reagents like enzymes, antibodies, antigens etc., and liquid samples in more than a single well at the same time. It can also be used to immobilize these highly charged molecules on a surface of a well by means of adsorbtion.

DESCRIPTION

The technique is based on electrospray. The setup for one capillary is shown in Figure 1.

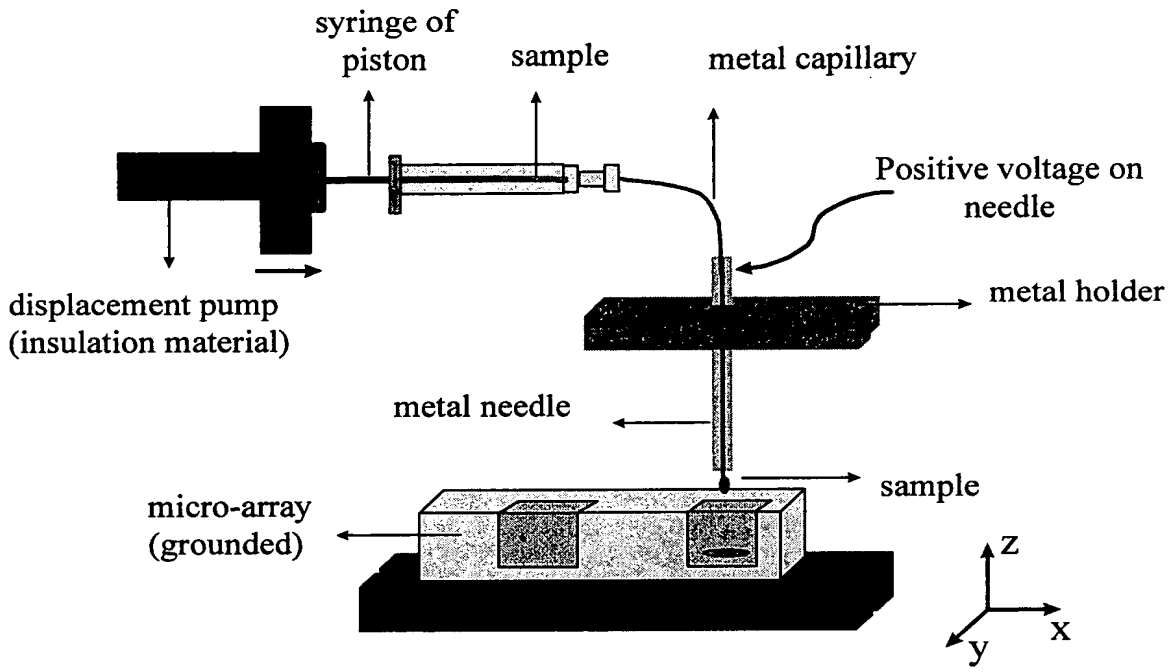

Figure 1: electrospray setup for the injection of less than 100 picoliters per second in 0.8-1.6 nanoliter wells by using one capillary.

A silicon micro-array of 25 wells is formed by means of anisotropic etching. This micro-array is grounded by attachment to a ground electrode of a *HCN 12500 power supply*. A metal capillary is placed in a needle and the capillary tip is placed vertically above one of the wells and connected to a positive electrode of the *HCN power supply*. The inner diameter of the capillary is 60 μm and the outer diameter is 160 μm. The capillary is connected to a 25 microliter syringe which contains the liquid. The piston of the syringe is moved by a *Harvard 2000 infuse pump* and the liquid is pushed through the capillary. At the same time a voltage difference of 1.5-2 kilovolt is applied between the capillary tip and the micro-array. The voltage difference creates an electric field between the liquid at the capillary tip and the well. When this force overcomes the surface tension of the droplet, the droplet will change in a cone with a jet. The cone formation and the dimensions are given in Figure 2:

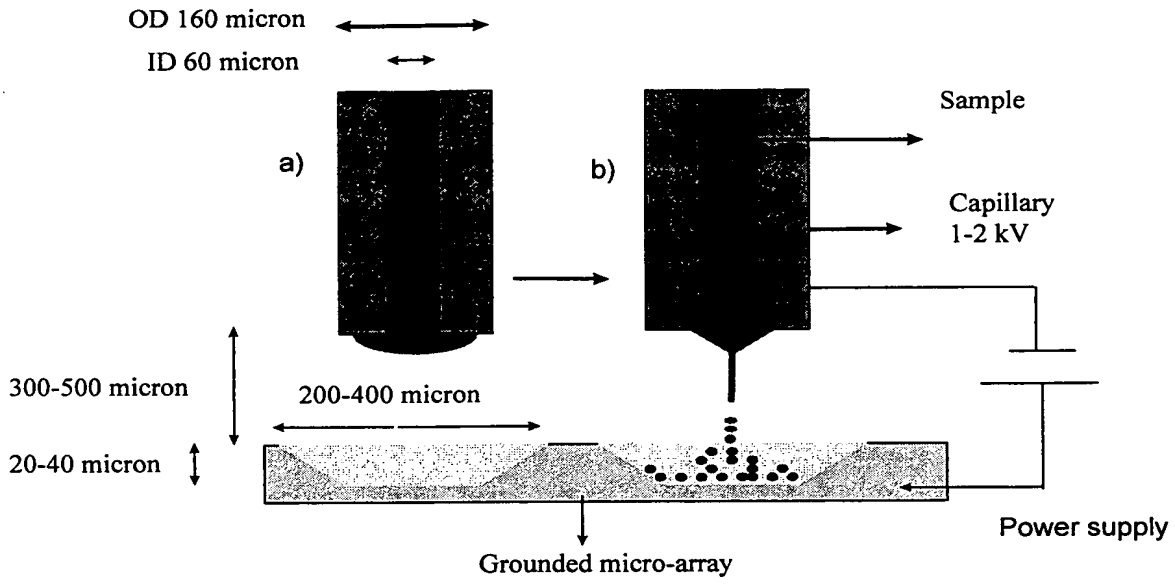

Figure 2: close view of the capillary tip and micro-array dimensions : a) droplet formation at the end of capillary tip without potential difference, b) potential difference resulting in formation of a liquid cone and breakup of the jet into small droplets (nanometer diameter).

Dimensions

The wells have a diameter of 200-400 micrometer and a depth of 20-40 micrometer. The wells are squared or circular shaped. Flat surfaces with reactive sites can also be used. It is also possible to make the surface of the well of silicium dioxide, gold or another metal by means of chemical vapor deposition.

The distance between the capillary tip and well surface is varied between 250 and 500 micrometer depending on the spray-area that is needed.

At the moment a metal capillary is used with an inner diameter of 60 micrometer and an outer diameter of 160 micrometer. In the future capillaries with smaller inner and outer diameter will be used.

Liquid handling Device

A liquid handling device will be developed in the future which allows for the filling of 100 or more wells, with different liquids, in less than 10 seconds. A possible design is described in Figure 3:

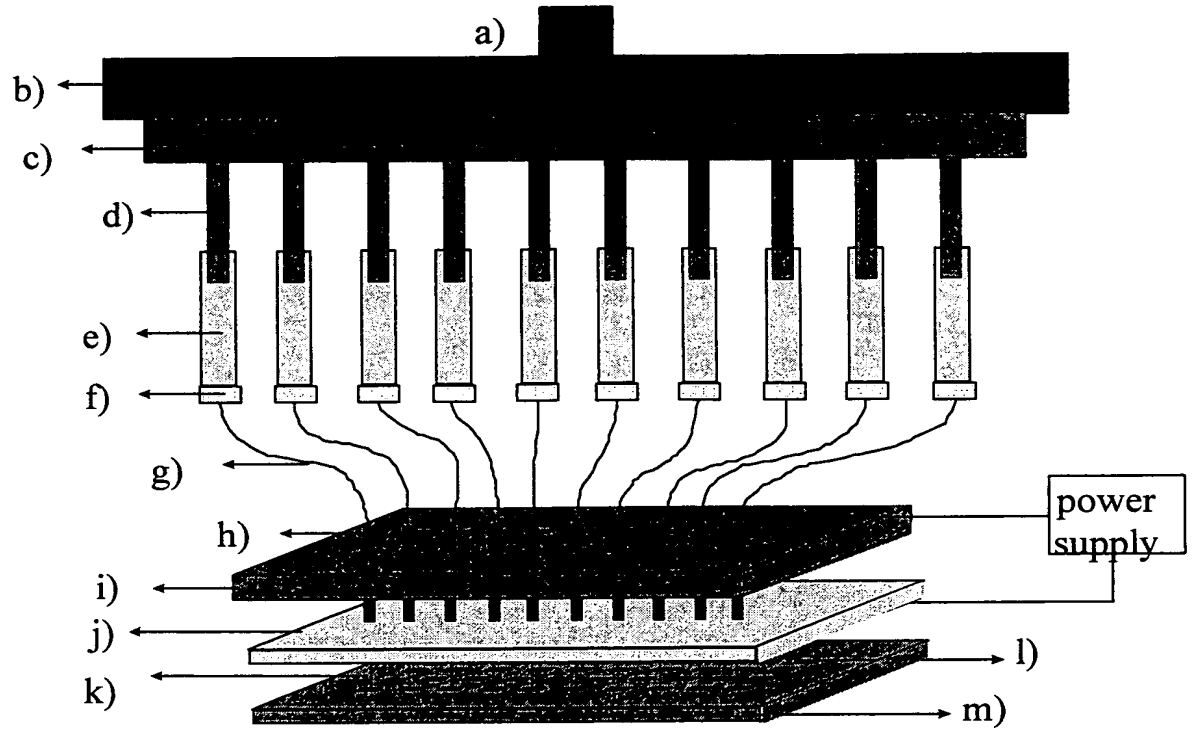

Figure 3 : liquid handling device which allows for filling of a total micro-array with different liquids in a few seconds

The pusher block (b)is moved by the high precision Harvard 2000 motor. The smallest possible flowrate the Harvard pump can handle is 0.0001 microliters per hour (0.033 picoliters per second). The plunjers (d) that are connected to the plunjer block (c) push the liquid through the syringes and then through the metal capillaries. The capillaries (k) are attached to the syringes with special made adaptors (f). The capillaries (flexible) are fixed in a delrin block (insulation material) in order to insulate them from each other and furthermore, to position them precisely above the wells (l). The capillary tips have to be spaced at a certain distance from each other to avoid crosstalk of the electric fields. The tips of the capillaries are placed above the wells at a distance of 250-500 micrometers depending on the desirable spraydiameter.

A certain voltage difference is applied between the capillaries and the wells of the micro-array. As the liquids reach the tips of a capillaries, the liquids are sprayed in the 100 or more wells for a certain time (depending on the desirable amount). Then the power supply is turned off and the filled micro-array is exchanged for a new micro-array and the power supply is turned on again.

a) Connection between motor and pusher block
b) Pusher block
c) Plunjer block that can be replaced
d) Plunjers (metal pins) that are soldered on the plunjer block

e) Syringes containing different liquids
f) Capillary adaptor
g) Capillary
h) Metal needle to position the capillary above the well
i) Metal plate with holes wherein the needles are placed
j) Silicium micro-array with wells
k) Well

Claims so far

The new developed method comprises a micro-injection technique which is based on the electrospray principle. The method allows for

1 : dispensing of liquids in sub-nanoliter up to microliter wells, and dispensing on a flat surface of a micro-array with flowrates lower than 100 picoliters per second

2 : dispensing liquids in such a way that a thin layer of small droplets covers the surface of a well or the surface of a flat plate.

3 : the diameter of this thin layer can be adjusted by changing the distance of the capillary tip and the well.

4 : creating a spray of which the droplet size is in the order of a few nanometers and is variable.

5 : charging enzymes, antibodies and antigens in order to immobilize these molecules by means of adsorption on the well surface, or by means of covalent attachment with carboxylgroups which are immobilized on the well surface.

6: dispensing in more wells of an array (25-1000) at the same time by using more than one capillary. Every capillary can be filled with a different liquid which allows for filling of every single well with a different liquid.